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Research Article

Potentiality of Formulated *Pseudomonas fluorescens* and *Bacillus subtilis* for the Management of Sheath Blight Disease of Rice Under Field Condition

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ABSTRACT

Rice production is severely affected by sheath blight disease in Bangladesh which is caused by a fungus *Rhizoctonia solani* that is still merely managed by synthetic chemical fungicides. In this study, different isolates of *Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated to assess their inhibitory effects against *R. solani* both *in- vitro* and field condition following dual culture method in order to formulate technique for the management of sheath blight of rice. Among all the isolates, *P. fluorescens* (Pf- 7 and Pf- 8) and *B. subtilis* (Bs-17 and Bs-21) showed the highest *in –vitro* mycelial growth suppression of the pathogen. Based on the *in-vitro* mycelial growth suppression performance, fourteen treatments combinations, either single or in consortia of antagonistic bacteria in talc-based formulations were applied by root dipping. All the formulated bacterial isolates showed significant reduction in disease and severity compared to untreated control and chemical fungicide. At 90 DAT, both the disease incidence and severity were found lowest in T_{11} (31.67 % and 37.33 % respectively) where the seedlings were treated for 24 hours with consortium of Pf-7 + Pf- 8 formulated in talc followed by in T_{12} (32.00 % and 38.00 %) where the seedlings were treated for 24 hours with consortium of Bs-17 + Bs-21 formulated in talc respectively. Vegetative and yield parameters were also significantly increased in both T_{11} and T_{12}. The highest yield as well as Benefit Cost Ratio (BCR) were found in T_{11} (2.50 kg/ m^2 and 2.20) followed by T_{12} (2.49 kg/m^2 and 2.18). Thus, the consortia of bacterial isolates (*Pseudomonas fluorescens* and *Bacillus subtilis*) with seedling dipping time for 24 hour represents a promising alternative to chemical fungicides for the management of sheath blight of rice in field condition.

Introduction

Grown over millions of hectares across Asian countries, rice is an economical crop for farmers and labourers and is a major staple food (Zibaee, 2013). Numerous fungal, bacterial, nemic and viral diseases can harm rice crops leading to about 40% yield loss (Srinivasacharya et al., 2002). Sheath blight (ShB) caused by *Rhizoctonia solani* an important fungal disease of rice in all rice cultivating areas worldwide because of its significant effects on yield loss (Prasad and Eizenga, 2008).In Bangladesh,Sheath blight of rice causes yield losses of about 35% in conducive condition(Khatun et al., 2021) and worldwide yield losses is about 4-50 %which depends on some factors related to host, pathogen as well as climatic condition (Kumar et al., 2020).But the production of rice needs to be increased to cater its ever increasing populations of Bangladesh (Barois et al., 2024).So, it is imperative to avoid all the biotic impediments including diseases to maximize the yield of rice.

R. solani is a soil-borne pathogen and mostly it can survive for long time in crop residues, soil organic matter or transforming into dormant structures like sclerotia (Panth et al., 2020). Due to its wide host range, eradication or management of soil borne pathogens is very tough. The application of fungicides and fumigants or combination of resistant cultivars and fungicides are commonly used ways to suppress sheath blight disease. It should be mentioned that there are clear problems with the use of fungicides, such as lead to development of fungicide resistance, ozone layer depletion, destruction of aquatic ecosystems, ecological disruption, and risks to human health (Christopher et al., 2010).Due to lack of complete resistant cultivar, the plantation of the cultivars alone has no significance to reduce the disease (Liu et al., 2013).The recent works on the management of various plant disease have been focusing on the development of environmentally safe methods. As a result, in the recent years, the importance of finding alternatives to reduce the usage

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of hazardous agrochemicals has increased (Boukaew et al., 2013). Reducing the need for chemicals in disease management is possible with the implementation of biocontrol management of plant diseases (Han et al., 2015; Nirmalkar et al., 2017). The application of biological agents not only reduces the plant diseases but also helps in the growth promotion of crops (Thakur et al., 2022). Plant growth promoting rhizospheric-bacteria (PGPR) play an important role against the wide range of pathogens to prevent disease development. They are capable to promote plant growth as well as reduce pathogenic activity *via* production of different phytohormones, atmospheric nitrogen fixation, antagonism, competition, ACC deaminase activity, induced systemic resistance and siderophore production (Gómez-Lama Cabanás et al., 2014). Among several PGPR, systemic defence against sheath blight is effectively provided by *Bacillus and Pseudomonas*. Both genera of PGPR are highly available in soil and may easily show antimicrobial behaviour through the association with plant system (Dimkić et al., 2022).*Pseudomonas fluorescens* isolates increased the expression of the NPR1 and phenylalanine ammonia lyase (PAL) genes, the production of peroxidase and polyphenol oxidase enzymes, cytokinin hormone and antibiotics, which may be related to a decrease in disease incidence as well as strengthening the plants' inherent defence mechanisms (Jain & Das, 2016; Elsharkawy et al.,2022). Again, the growth of mycelia of *R. solani* is significantly reduced by *Pseudomonas fluorescens* strains through increasing chitinase activity under both *in vitro* and field condition (Radjacommare et al., 2002). On the other hand, *Bacillus subtilis* also produces antibiotics and secondary metabolites those are crucial for both controlling plant diseases and supporting plant development (Glick, 1995). Notable evidences are reported about rhizospheric bacterial organism like *B. subtilis*, which significantly impact on *R. solani* by restricting their growth and sclerotial development (Luo et al, 2005). Moreover, *Bacillus subtilis* is capable of reducing of pathogen inoculum at the infection site as well as enhancing plant growth through suppressing *R. solani* because of antibiosis, lysis of pathogen hyphae, parasitism and competition for nutrients and space (Luo et al, 2005; Wang et al., 2009) which ultimately helps in the increased production of rice crops (Kumar et al.,2012). Thakur et al. (2022) reported that among the isolates of *B. subtilis* against *R. solani*, BS4 showed lowest disease index percentage compare to control.

The active effects of both *Pseudomonas* and *Bacillus* on plant growth led to tremendous interest in using them as a biological agent for the biocontrol of plant diseases those area great alternative to chemicals (Sivakumar and Narayanaswamy, 1998; Dimkić et al., 2022). Many researchers worked with different bioagents for the management of sheath blight. Yet now, any works were not reported in Bangladesh to evaluate these bioagents (*Pseudomonas fluorescens* and *Bacillus subtilis*) against the disease along with taking concerns of root dipping time. This study was aimed to assess the efficacy of formulated products of *P. fluorescens* and *B. subtilis* against sheath blight of rice in field condition.

Materials and Methods

Source of Rhizoctonia solani and its multiplication

The causal organism of sheath blight of rice, *R. solani* was collected from the Probiotics and Bioformulation Lab, Dept. of Plant Pathology, Bangladesh Agricultural University, Mymensingh. Initially, the pathogenicity test of *R. solani* was conducted on rice plants in the green house in order to confirm the ability of the pathogen causing rice sheath blight. For purification, the pathogen was transferred to fresh plates (Kazempour, 2004).

R. solani was sub-cultured in Potato Dextrose Agar (PDA) medium for multiplication and cultured for a week and kept at 4 °C for later use (Doley and Jite, 2012).

Collection and multiplication of biological agents

Isolates of *Bacillus subtilis* and *Pseudomonas fluorescens* were preserved in glycerin solution at -80 °C for future use which was previously characterized at the Probiotics and Bioformulation lab of the Department of Plant Pathology. For culturing *B. subtilis* and *P. fluorescens* from stock, a sterile tooth pick was inserted in the glycerin stock and immediately streak on nutrient agar and King's B medium respectively and subsequently incubated at 30±2 °C (Murray et al., 2003; Abdulkadir and waliyu, 2012).

Assessment of mycelial growth inhibition of Rhizoctonia solani

Dual culture method was followed for assessing growth inhibition of *R. solani* by the bacterial isolates. The bacterial isolates were streaked separately in the center of Petri dishes, and a 9 mm mycelial block of *R. solani* was placed at one side of the same petri dish. The dishes were then incubated at 25±2°C for 7 days (Vidhyasekaran et al., 1997). The control treatment represents the culture of *R. solani* alone. Data on mycelial growth (mm) were collected at three, five and seven days after incubation (DAI) until the control plate was fully grown by the pathogen. The percent inhibition of pathogen growth was estimated following formula of Vincent (1927).

I = C - T / C x 100
Here,
I = Percent inhibition
C = Mycelial growth of the pathogen in control
T = Mycelial growth of the pathogen in dual plate

Preparation of bacterial suspension and consortia of P. fluorescens and *B. subtilis*

One loopful of fresh bacterial culture from best performing strains of *B. subtilis* (BS-17, BS-21) and *P. fluorescens* (Pf-7, PF-8) were inoculated into nutrient broth and King's B broth respectively, and incubated for 48 hs at 28 °C in a rotary shaker at 150 rpm. For preparing the bacterial consortia, at first two bacteria are cultured separately on king's B medium and Nutrient broth after incubation for 48 hs at 28 °C on a rotary shaker at 150 rpm (Jain et al*.,* 2015; Chakraborty et al*.,* 2021). Afterward, two broths were mixed and suspension containing $9x10^8$ CFU/mL was utilized to prepare talc-based formulation.

Preparation and preservation of talc-based formulations

The formulation was prepared following the method described by Vidhyasekaran and Muthamilan's (1995) with slight modification. To prepare the powder formulation, 400 mL of bacterial cells (9×10^8 CFU/mL) was added to a sterilized mixture including 10 g of carboxy methyl cellulose (CMC), 1 kg of talc, and 50 mL of 50 % glycerol. Additionally, 15 g $CaCO_3$was mixed to adjust the pH at 7.0. All the ingredients were thoroughly incorporated and air dried in a sterile environment.

Treatments of the experiment

The treatments were randomly assigned to the experimental units. Fourteen treatments (formulated in talc) were as follows:
T_0 = Control
T_1 = Seedling treatment with Pf-7 for 12 hs
T_2 = Seedling treatment with Pf-8 for 12 hs
T_3 = Seedling treatment with Bs-17 for 12 hs
T_4 = Seedling treatment with Bs-21 for 12 hs
T_5 = Seedling treatment with consortium of Pf-7 + Pf-8 for 12hs
T_6 = Seedling treatment with consortium of Bs-17+ Bs-21 for 12hs
T_7 = Seedling treatment with Pf-7 for 24 hs
T_8 = Seedling treatment with Pf-8 for 24 hs
T_9 = Seedling treatment with Bs -17 for 24 hs
T_{10} = Seedling treatment with Bs -21 for 24 hs
T_{11} = Seedling treatment with consortium of Pf-7+ Pf-8 for 24 hs
T_{12} = Seedling treatment with consortium of Bs-17+ Bs-21 for 24 hs
T_{13} = Carbendazim 50 WP

Field evaluation of the potential bacterial antagonists against R. solani

In this experiment, 35 days old rice seedling roots were dipped into a suspension containing 10 % of formulated products for overnight (Rabindran and Vidhyasekaran, 1996). For foliar application, @ 10 % talc- based formulation of bioagents were sprayed at 20, 40, 60 days after transplanting (DAT) on rice plants (Raj et al*.,* 2020). Data were collected on vegetative parameters (Plant height, No. of tillers/ hill, No. of leaves/ hill), yield parameters, (No. of panicles/ hill, Total no. of grains/ panicle, Panicle length, Thousand- grain weight and Yield). Moreover, disease incidence and severity of sheath blight was assessed according to James (1974) and Ahn and Mew (1986) respectively at 60 DAT, 75 DAT, 90 DAT. At last, benefit cost ratio (BCR) was calculated following method of Mehmood and Sabir (2011).

Statistical analysis

The data were analyzed following the analysis of variance (ANOVA) and mean differences were compared by Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984) at significant levels $P < 0.05$ with three replications for each treatments using Statistics 10 software. Completely Randomized Design (CRD) was applied for *In- vitro* tests and Randomized Complete Block Design (RCBD) for field experiments.

Results

In vitro screening of Pseudomonas fluorescens and Bacillus subtilis isolates

Most of the isolates of both bacterial bioagents reduced mycelial growth of *R. solani* at different days after incubation over control. According to last date of data collection, among 16 isolates of *Pseudomonas fluorescens,* Isolate Pf-7 showed highest mycelial growth suppression (73.12 %) over control. This was statistically similar to only Pf-8 (71.30 %) (Table 1).

Table 1. ***In-vitro*** **screening of** ***Pseudomonas fluorescens*** **isolates to suppress mycelial growth of** ***Rhizoctonia solani***

Name of isolates	Mycelial growth (mm)					
	3 DAI		5 DAI		7 DAI	
	Mean mycelial growth	% Inhibition	Mean mycelial growth	% Inhibition	Mean mycelial growth	% Inhibition
Pf- 10	34.00 ij	38.18	35.22 h	48.69	36.00 j	56.16
Pf- 14	40.67 f	26.05	42.67 e	35.14	44.33 fg	41.01
Pf- 6	36.00 ghi	34.54	37.67 fg	44.23	39.67 hi	49.49
Pf- 12	35.00 hij	36.36	36.67 gh	46.05	38.00 i	52.52
Pf- 3	32.67 j	41.81	34.33 h	50.30	35.33 j	57.38
Pf- 8	26.00 k	52.72	27.33 i	63.03	27.67 k	71.30
Pf- 7	24.33 k	55.76	26.00 i	65.45	26.67 k	73.12
Pf- 18	47.67 b	13.32	49.67 b	22.41	49.67 bc	31.30
Pf- 19	37.00 gh	32.72	38.67 fg	42.41	40.33 h	48.89
Pf- 11	43.33 de	21.21	45.00 de	30.90	45.33 ef	39.20
Pf- 5	38.00 g	30.90	40.00 f	40.00	42.67 g	44.03
Pf- 2	41.33 ef	24.85	44.33 de	32.12	46.67 de	36.76
Pf- 16	36.67 gh	33.32	38.67 fg	42.41	40.00 h	48.89
Pf- 15	41.33 ef	24.85	43.00 e	34.54	44.67 f	40.40
Pf- 17	46.56 bc	15.34	49.00 bc	23.63	50.67 b	29.49
Pf- 4	44.67 cd	18.78	46.67 cd	27.87	48.33 cd	33.74
Control	55.00 a	---	62.00 a	---	66.89 a	---
CV (%)	2.10	---	1.95	---	1.44	---

DAI = Days after incubation; CV= Co-efficient of Variation

Among nine *Bacillus subtilis* isolates, maximum mycelial growth suppression was observed in Isolate Bs-17 which was statistically similar to Bs-21. These two isolates suppressed 61.32 % and 60.70 % of mycelial growth respectively (Table 2).

Table 2. ***In vitro*** **screening of** ***Bacillus subtilis*** **isolates to suppress mycelial growth of** ***Rhizoctonia solani***

Name of isolates	Mycelial growth (mm)					
	3 DAI		5 DAI		7 DAI	
	Mean mycelial growth	% Inhibition	Mean mycelial growth	% Inhibition	Mean mycelial growth	% Inhibition
Bs- 8	29.67 d	46.05	31.00 d	49.73	31.67 d	54.46
Bs- 27	30.33 d	44.85	31.23 d	49.35	32.63 d	53.08
Bs- 15	32.00 d	41.81	33.33 d	45.95	33.67 d	51.58
Bs- 17	24.90 e	52.98	25.90 e	58.00	26.90 e	61.32
Bs- 21	25.86 e	54.72	26.57 e	56.91	27.33 e	60.70
Bs- 10	35.77 c	34.96	36.67 c	45.45	37.33 c	46.32
Bs- 31	36.77 bc	33.14	38.00 bc	43.03	39.00 bc	43.92
Bs- 26	35.33 c	35.76	36.43 c	45.89	37.33 c	46.32
Bs- 41	39.00 b	29.09	39.67 b	35.67	40.67 b	41.52
Control	55.00 a	---	61.67 a	---	69.55 a	---
CV (%)	2.69	---	2.64	---	2.11	---

DAI = Days after incubation; CV= Co-efficient of Variation

Effect of different treatments on disease incidence (DI) and disease severity (DS) of sheath blight of rice in field condition

Effect of different treatments on percentage of disease incidence (DI) and severity (DS) of sheath blight in the field condition are presented Figure 1 and 2. Both diagrams indicated that DI and DS percentage were lower when plants treated with bioagents compare to control. The ranges of DI at different days after intervals (60,75 and 90 DAT) were 26.00 % to 51.00 %, 30.00% to 65.00% and 31.67 % to 75.00 % respectively. At 90 DAT, T_{11} showed the lowest disease incidence (31.67 %) followed by T_{12} (32.00 %) while the control treatment provided the highest value (Figure 1). Similarly, the ranges of DS at different days after intervals (60,75 and 90 DAT) were 33.33 % to 58.00 %, 35.00 % to 75.00 % and 37.33 % to 80.00 % respectively. T_{11} exhibited the lowest severity percentage in all three-time period. T_{12} also exhibited lower severity and this was statistically similar to T_{11}. On the other hand, the control treatment (T_0) exhibited highest value (Figure 2).

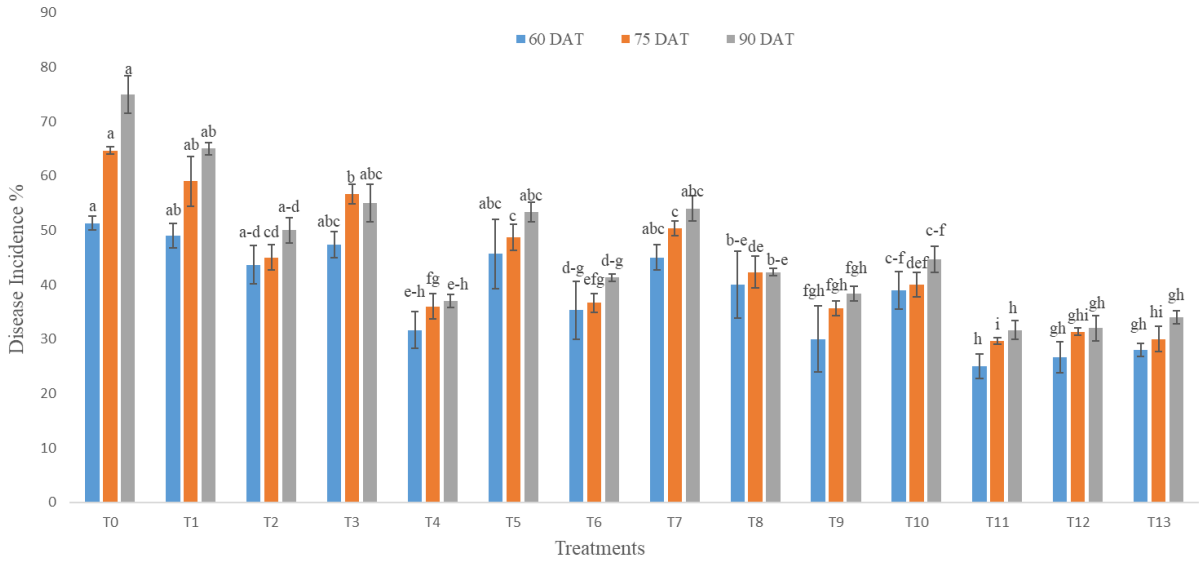

Figure 1. Effects of different treatments on disease incidence (%) of sheath blight of rice at various DAT (Days After Transplanting) in the field condition

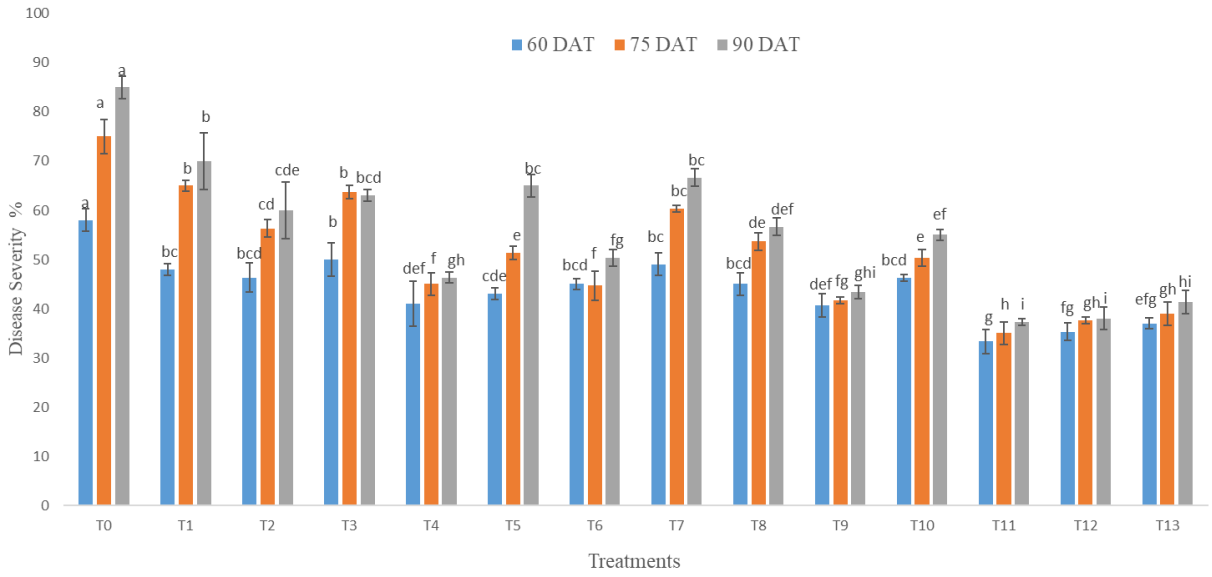

Figure 2. Effects of different treatments on disease severity (%) of sheath blight of rice at various DAT (Days After Transplanting) in the field condition

Effects of different treatments on vegetative parameters of rice in field condition

Plant height

Promotion of plant height at different days after transplanting was observed (Table 3 and 4). AT 45 and 60 DAT, the highest plant height 39.55 cm and 58.33 cm were observed in T_{11}. Statistically similar plant height was observed in T_{12} at 45 and 60 DAT, which was 38.78 and 57.77 cm respectively (Table 3 and 4).

Table 3. Effects of different treatments on vegetative parameters of rice against sheath blight disease in the field at 45 DAT

Treatments	Plant height (cm)	No. of tillers/hill	No. of leaves/hill
T_0	28.89 i	5.00 i	35.33 h
T_1	31.44 h	15.44 b	38.22 g
T_2	33.78 defg	14.33 b	44.55 cd
T_3	32.55 gh	7.55 fgh	37.55 g
T_4	36.11 bc	12.33 c	46.66 b
T_5	33.33 efg	10.11 d	40.33 f
T_6	35.44 bcd	8.33 ef	39.22 fg
T_7	33.00 fgh	9.00 de	42.33 e
T_8	34.33 def	6.77 gh	42.89 de
T_9	36.77 b	7.66 fg	45.22 bc
T_{10}	35.00 cde	6.33 h	45.33 b
T_{11}	39.55 a	18.44a	49.33 a
T_{12}	38.78 a	18.33 a	48.89 a
T_{13}	36.33 bc	14.77 b	46.00 bc
CV (%)	1.68	3.88	1.53

CV= Co-efficient of Variation

Table 4. Effects of different treatments on vegetative parameters of rice against sheath blight disease due to effect of different treatments in the field at 60 DAT

Treatments	Plant height (cm)	No. of leaves/ hill	No. of tillers/hill
T_0	46.33 i	48.22 g	18.22 h
T_1	48.11 h	51.00 f	24.33 d
T_2	49.55 g	50.44 f	20.22 g
T_3	51.22 f	53.22 d	25.11 cd
T_4	50.33 fg	56.66 b	26.22 b
T_5	52.33 e	52.33 de	20.55 g
T_6	55.44 b	51.33 ef	23.11 e
T_7	53.33 d	56.33 b	26.11 bc
T_8	54.22 cd	54.55 c	23.22 e
T_9	55.22 b	57.22 c	25.33 bcd
T_{10}	50.44 fg	55.22 cd	21.77 f
T_{11}	58.33 a	59.33 a	29.22 a
T_{12}	57.77 a	58.55 a	28.33 a
T_{13}	55.00 bc	56.44 b	26.00 bc
CV (%)	0.61	0.68	1.51

CV= Co-efficient of Variation

No. of tillers/hill

Variation in no. of tillers/hill observed in different treatments. At 45 and 60 DAT, control treatment showed 5.00 and 18.22 tillers/hill respectively. The highest no. of tillers was found in T_{11} which had about 3 times more tillers at 45 DAT and 60% more tillers at 60 DAT compared to control treatment. Statistically similar and the higher no. of tillers was also found in T_{12} (18.33 and 28.33) at 45 and 60 DAT respectively (Table 3 and 4).

No. of leaves/hill

The highest no. of leaves/hill was observed in T_{11} and T_{12} with 49.33 and 48.89 leaves respectively at 45 DAT whereas at 60 DAT, the highest no. of leaves/hill was 58.85 and 59.33 respectively. The lowest no. of leaves/hill was observed in control which was 35.33 and 48.22 leaves/ hill at 45 and 60 DAT respectively (Table 3 and 4).

Effects of different treatments on reproductive parameters of rice in field condition

No. of panicles/ hill

The lowest no. of panicles (17.76 panicles/ hill) was observed in control at 120 DAT, whereas at 120 DAT the highest panicles number/hill was recorded in T_{11} (29.22) and T_{12} (28.66) which were 65.0 % and 62.28% higher respectively compared to control treatment (Table 5).

Table 5. Effects of different treatments on reproductive parameters of rice against sheath blight disease in the field at 120 DAT

Treatments	No. of panicles /hill	Total no. of grains/panicle	Panicle length (cm)	Thousand grains weight (gm)	Yield (kg/m²)	Benefit Cost Ratio (BCR)
T_0	17.66 i	155.22 g	18.11 f	20.50 g	1.48 ij	0.90j
T_1	19.33 h	160.11 ef	20.66 ef	25.55 cde	1.84 fg	1.75d
T_2	21.33 g	164.11 cd	27.11b	28.16 b	2.20 b	1.65f
T_3	22.11 g	168.56 b	25.00 bc	25.90 cd	2.22 bc	1.63fg
T_4	27.11 b	170.56 b	27.11 b	26.83 bcd	2.00 cd	1.88bc
T_5	25.00 de	165.78 c	21.77 de	24.26 ef	1.79 h	1.72e
T_6	26.22 c	158.56 f	23.67 cd	28.40 b	1.88 de	1.60h
T_7	20.11 h	162.11 de	25.11bc	23.50 f	1.83 f	1.89 b
T_8	24.22 e	160.56 ef	23.56 cd	25.46 de	1.89 d	1.55 i
T_9	23.33 f	165.56 c	26.11 bc	27.73 b	1.79h	1.85 c
T_{10}	25. 22 d	166.22 c	24.22 cd	27.16 bc	1.76 hi	1.70 ef
T_{11}	29.22 a	173.22 a	30.11 a	31.56 a	2.50 a	2.20 a
T_{12}	28.66 a	172.89 a	29.89 a	30.73 a	2.49 a	2.18 a
T_{13}	27.00 bc	169.56 b	25.67 bc	27.83 b	2.11 b	1.87 bc
CV (%)	1.16	0.43	3.51	2.02	14.55	17.88

CV= Co-efficient of Variation

Panicle length

The highest panicle length was found in T_{11} (30.11 cm) and T_{12} (29.89 cm) respectively which were statistically similar to each other. These treatments had 66.26 % and 65.04 % higher panicle length than control (Table 5).

Total no. of grains/panicle

Total no. of grains/panicle significantly varied among the treatments. The lowest no. of grains/panicle (155.22) was observed in control whereas the highest no. of grains/panicle was observed in T_{11} (173.22). Statistically similar and the higher no. of grains/panicle was also found in T_{12} (172.89) (Table 5).

1000 grain weight

Different treatments showed significant variation for 1000 grains weight. The highest 1000 grain weight was found in T_{11} (31.56 g) and T_{12} (30.73 g) which were 55.41 % and 40.90 % higher than control respectively (Table 5).

Yield and Benefit cost ratio (BCR)

The highest yield in unit area (1 m^2) was recorded in T_{11} and T_{12} (2.50 and 2.49 kg/m^2) which was 68.24 %- 68.91 % higher than the control treatment. The BCR was highest in T_{11} (2.20) and T_{12} (2.18) which was 2.4 times higher than the control treatment (Table 5).

Discussion

A total sixteen *Pseudomonas fluorescens* and nine *Bacillus subtilis* isolates were collected and evaluated for their growth suppressing efficacy of *Rhizoctonia solani*, the causal organism of sheath blight of rice. Among these isolates, *P. fluorescens* (Pf-7 and Pf-8) as well as *B. subtilis* strains (BS-17 and BS-21) exhibited the highest suppression of mycelial growth. This finding is consistent with the findings of Nagendran et al. (2019), who reported that certain strains of *P. fluorescens* and *B. subtilis* suppressed the mycelial growth up to 75 % and 51 % under laboratory condition. In another study conducted by Raj et al. (2020), they also reported that *B. subtilis* Bs-1 isolate showed the highest growth inhibition (60.20 %) of *R. solani*. Many researchers stated in their multiple studies that both *P. fluorescens* and *B. subtilis* isolates can impede the mycelial growth of the pathogen due to having their capacity to produce some enzymes, VOCs, peptides and antibiotics (Safdarpour and Khodakaramian, 2018; Carmona-Hernandez et al., 2019).

In field experiment, T_{11} and T_{12}, consisting of consortium of bacterial strains exhibited significantly better result in disease suppression compare to all treatments. In some cases, the consortium showed inconsistent performance than individual treatments. Besides, the consortia of both bacterial genera showed different performances. It may be hypothesized that due to short dipping time, the bacterial strains could not interact with themselves as well as they might not effectively penetrate into the root cells. Nevertheless, Sang et al., (2007) reported that plant root dipping application of bacterial strains before transplanting is very effective technique against the soil-borne pathogens. On the other hand, Nandakumar et al. (2001) stated that consortium of two strains of *P. fluorescens* had a lower disease index (33.3 %) than their individual performance (40 % - 42 %) against sheath blight of rice. Ali and Nadarajah (2013) and Khedher et al. (2015) who also described that combine application of *B. subtilis* and *Trichoderma* showed

minimum disease incidence (11 %) and disease severity (4.33 %) compare to control against *Rhizoctonia solani.*

Besides disease suppression, T_{11} and T_{12} promoted the highest growth and reproductive parameters of rice plants. These results are supported by the findings of Nagendran et al. (2013) and Liu et al. (2018). Similarly, Kumar et al. (2017) also proved that plots treated with *B. subtilis* UASP17 showed higher yield compare to control. It may be due to their capacity to produce IAA, siderophore, atmospheric nitrogen fixation, solubilize phosphate by both antagonist strains which helps to enhance plant growth when they are used as biological agents (Karnwal and Mannan, 2018; Jamali et al., 2020). IAA promotes optimum root growth and elongation and thus, benefiting the plants to uptake sufficient water as well as nutrients (Nascimento et al., 2014). Phosphate is a major essential nutrient for plants but they remain as unavailable forms (Bakhshandeh et al., 2015). Several theories regarding the superior performance of consortium were provided by researchers, i.e.: diversified mechanisms offered by antagonists in consortium, broad range of target pathogen, synergistic relation among compatible members of consortium (Niu et al., 2020). Besides degrading cell wall of pathogen, early expression of peroxidase, chitinase and β 1, 3 glucanase by *P. fluorescens* and *B. subtilis* provide plants with induced systemic resistance (ISR) against pathogenic disease (Jamali et al., 2020;Nur Mawaddah et al., 2023). As defense enzymes, peroxidase assists in wound healing, suberification and lignification and PAL is involved in synthesizing defense chemicals like lignin & phenol, trigger Salicylic acid defensive pathways (Elsharkawy et al., 2022). Both of the antagonists are also reported to produce biofilm (Rafique et al., 2015) which forms between lesion and pathogens and assists in preventing contact of host and pathogen. The superiority of *P. fluorescens* over *B. subtilis* may be explained by the fact that *P. fluorescens* is capable of efficiently colonizing on both phyllosphere and rhizosphere where the pathogen mainly attacks (Haque and Khan, 2021).

Conclusion

Pseudomonas fluorescens (Pf-7, Pf-8) and *Bacillus subtilis* (Bs-17, Bs-21) were highly effective for managing the sheath blight of rice when seedlings were dipped into formulated suspension for 24hs. Performances of consortia of the antagonistic bacteria along with higher root dipping time were much greater than their individual application through talc-based formulation in field condition. This study highlights the potential benefits of using bacterial consortia as well as effect of different root dipping time against *R. solani* and suggests that further research is required to explore their effectiveness across multiple locations and combinations for eco-friendly management of sheath blight of rice.

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